

# BMJ Open Diagnostic and prognostic accuracy of miR-21 in renal cell carcinoma: a systematic review protocol

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## ABSTRACT

**Introduction:** Renal cell carcinoma (RCC) is the most common neoplasm in adult kidneys. One of the most important unmet medical needs in RCC is a prognostic biomarker to enable identification of patients at high risk of relapse after nephrectomy. New biomarkers can help improve diagnosis and hence the management of patients with renal cancer. Thus, this systematic review aims to clarify the prognostic and diagnostic accuracy of miR-21 in patients with RCC.

**Methods and analysis:** We will include observational studies evaluating the diagnostic and prognostic roles of miR-21 in patients with renal cancer. The index test and reference standards should ideally be performed on all patients. We will search PubMed, SCOPUS and ISI Web of Science with no restriction of language. The outcome will be survival measures in adult patients with RCC. Study selection and data extraction will be performed by two independent reviewers. QUADAS-1 will be used to assess study quality. Publication bias and data synthesis will be assessed by funnel plots and Begg's and Egger's tests using Stata software V.11.1.

**Ethics and dissemination:** No ethical issues are predicted. These findings will be published in a peer-reviewed journal and presented at national and international conferences.

**Trail registration number:** This systematic review protocol is registered in the PROSPERO International Prospective Register of Systematic Reviews, registration number CRD42015025001.

## INTRODUCTION

Renal cell carcinoma (RCC) is the most common neoplasm in adult kidneys.<sup>1</sup> The incidence of RCC is increasing by approximately 20% per annum.<sup>2</sup> Distinguishing the RCC subtypes is of clinical importance because they have different prognoses and subsequently require different management plans.<sup>3</sup> The most common form of RCC is the clear subtype RCC (ccRCC) that accounts for 75%–80% of cases; other less common subtypes include papillary RCC

## Strengths and limitations of this study

- This systematic review, for the first time, will evaluate the prognostic and diagnostic accuracy of miR-21 in patients with renal cell carcinoma through a search of several databases without placing restrictions on language.
- The study screening, data extraction and assessment of risk of bias of the current study will be conducted by two researchers independently.
- We expect some potential heterogeneities between previous studies, including stage and histological grade in patient samples.

(pRCC), chromophobe RCC (chRCC) and collecting duct carcinoma.<sup>4</sup> Although surgery is curative for the localised disease, a significant proportion of patients relapse or metastasise. Metastatic RCC is difficult to treat. The 5-year survival rate for metastatic RCC is dismal at less than 10%.<sup>5</sup> At present, there is an absence of biomarkers for the early detection and follow-up of the disease, which is responsible for the late diagnosis and the subsequent poor prognosis.<sup>6</sup>

Factors influencing RCC prognosis can be classified into anatomic, histological, clinical and molecular.<sup>2</sup> Pathological stage, based on the size and extent of invasion by the tumour, is the most accurate indicator of prognosis.<sup>7</sup> Recent evidence shows that molecular signatures can classify RCC subtypes more accurately than morphological characteristics.<sup>8–10</sup> Micro RNAs (miRNAs) are small non-coding RNA nucleotides that post-transcriptionally regulate the expression of their target proteins.<sup>4</sup> The first report suggesting a role of miRNAs in cancer was published in 2002.<sup>11</sup>

miRNAs are actively involved in kidney cancer pathogenesis.<sup>12–13</sup> Recently, the clinical implications of miRNAs in RCC have been systematically reviewed;<sup>14</sup> moreover, the potential roles of miRNAs as prognostic and

diagnostic tools in RCC as well as its subtypes have been reported in several studies.<sup>4 15–34</sup>

Among the numerous miRNAs, miR-21 has received specific attention because of its relationship with multiple cancers. It is upregulated in several tumours, including cancers of the breast, lung, colon, pancreas, prostate and stomach.<sup>35–38</sup> The effect of miR-21 on carcinogenesis and tumour progression has been experimentally analysed.<sup>39</sup> miR-21 regulates epithelial–mesenchymal transition phenotype and hypoxia-inducible factor-1 $\alpha$  expression in breast cancer stem cell-like cells and contributes to cell migration in metastasis.<sup>40</sup> The global prediction role of miR-21 for survival in patients with various carcinomas has been summarised in a meta-analysis.<sup>41</sup>

To date, several researchers have published their data on the prognostic value of miR-21 in RCC and raised concerns about its prognostic accuracy as a biomarker; however, these issues remain questionable.<sup>4 17 19 31</sup> This systematic review aims to evaluate the diagnostic and prognostic accuracy of miR-21 in patients with RCC.

## OBJECTIVES

The primary objectives are to determine the diagnostic and prognostic accuracy of miR-21 in patients with RCC. The secondary objectives include determining the diagnostic and prognostic accuracy of miR-21 in RCC subtypes together with the evaluation of heterogeneity and its potential sources in primary studies.

## METHODS

The methods adopted for this systematic review have been developed in accordance with the guidelines detailed on the PRISMA checklist. In addition, the PRISMA Flow Diagram will be employed to describe the flow of information through the different phases of this systematic review.<sup>42</sup> The protocol of this systematic review has been registered in PROSPERO 2015 (registration number: CRD42015025001). The Preferred Reporting Items for Systematic reviews and Meta-Analyses for Protocols 2015 (PRISMA-P 2015) have been used for preparing and reporting the protocol of this systematic review.<sup>43</sup>

## INCLUSION CRITERIA

### Types of studies

Observational studies (cross-sectional, case–control and cohort) will be included. Studies assessing animal models, review articles, proceedings, conferences and case series on patients dead from RCC will be excluded.

### Participants

Adult patients of both sexes with a diagnosis of RCC will be included. Tumour classification and stage will be diagnosed according to the TNM system and 2004 WHO classification.<sup>44 45</sup>

## Index test

### miR-21

miRNAs are highly conserved small non-coding RNA strands, approximately 22 nucleotides in length, that post-transcriptionally regulate gene expression and appear to be modulators of urological cancers.<sup>46 47</sup> miR-21 is one of the most frequently studied cancer-related miRNAs and is dysregulated in most cancers by acting as an oncogene.<sup>48</sup>

RCC considerably exploits the upregulation of miR-21 in comparison with healthy kidneys.<sup>4 39</sup> A significant difference exists between the expression levels of miR-21 and RCC subtypes with respect to the highest levels in ccRCC and pRCC subtypes. miR-21 levels are associated with higher stage and grade, and hence its expression level can be used as a diagnostic marker in distinguishing RCC subtypes.<sup>4</sup> The expression level of miR-21 is correlated with a span of 5-year survival and pathological stage in RCC.<sup>49</sup> Patients who are miR-21 positive have statistically shorter disease-free survival (DFS) and overall survival (OS) rates.<sup>4</sup> In the current review, miR-21 detection in RCC tissues will be defined on the basis of quantitative real-time PCR (qRT-PCR). There are several steps for miR-21 detection in RCC tissues based on qRT-PCR, which are as follows:

1. Total RNA is extracted from RCC samples (formalin-fixed, paraffin-embedded tissues or serum) using commercial kits, according to the manufacturer's protocol.
2. RNA concentrations and A260/280 ratio with a Nano Drop are determined.
3. The resulting miRNA for qRT-PCR is retained, and miRNA expression is measured by TaqMan miRNA assays.
4. Reverse transcription of miR-21 with a specified amount of total RNA using the TaqMan MicroRNA-specific Reverse Transcription Kit is performed.
5. The cycling conditions for miR-21 are selected according to the manufacturer's protocols.
6. All reactions are performed in triplicates.
7. The values of miR-21 with respect to a small nuclear RNA as a reference gene are normalised.
8. The relative overexpression or underexpression of miR-21 in patient samples is compared to that in normal subjects by the  $\Delta\Delta C_t$  method.<sup>4 7 19 31</sup>

## Gold standards

Studies will be eligible for this review if the clinical diagnostic criteria recommended by the WHO classification and TNM system of the renal tumours of adults are used as primary reference standards. The pathological diagnosis of RCC is recommended for all renal masses existent in radical nephrectomy or biopsies. Since clinical practice guidelines are routinely updated, we will use the criteria of reference standard that the primary studies adopted. Survival measures such as DFS, OS, recurrence-

free survival (RFS) and HR with 95% CI will be used as reference standards for prognostic accuracy.

### Target conditions

The target conditions for diagnostic and prognostic values will be verified by RCC diagnosis and survival measures, respectively.

### Outcomes

The primary outcomes will be all survival measures of patients with RCC. The secondary outcomes will include the determination of prognostic accuracy of miR-21 in different subtypes of renal cancer, as well as the determination of heterogeneity and its potential sources.

## SEARCH METHODS FOR IDENTIFICATION OF STUDIES

### Electronic searches

We will search the following databases: PubMed, Web of Science and Scopus, till 31 July 2015.

### PubMed search strategy

MeSH tags were found in PubMed. The details of the PubMed database search strategy and syntax are sequentially provided below.

1. miR21
2. miR-21
3. microRNA-21
4. miRNA-21
5. hsa-mir-21
6. 1 or 2 or 3 or 4 or 5
7. Renal
8. Kidney
9. 7 or 8
10. 6 and 9

[miR21 (tiab) OR microRNA-21(tiab) OR miRNA-21 (tiab) OR hsa-mir-21(tiab) OR miR-21(tiab)] AND [kidney (tiab) OR renal (tiab)].

### Other resources

Reference lists of relevant primary studies, reviews and key journals will be searched for additional studies.

### Data collection and analysis

A data extraction form will be developed, and study data will be independently assessed and extracted by two reviewers, AR and RR.

The following data will be extracted from all the included studies:

1. Study characteristics (author, year of publication, study design, setting, locations and patient enrolment strategies).
2. Test characteristics (test type, test condition, prespecified cut-off values, sampling protocol and criteria for test positivity).
3. Reference standards (histopathological diagnosis or criteria of the guideline used).

4. Participants' characteristics (age, gender, ethnicity, disease type, tumour sizes and tumour stages).
5. Statistics for meta-analysis, including true positives, false positives, true negatives, false negatives, sensitivity, specificity, positive and negative likelihood ratios, area under the curve (AUC) for diagnostic value, OS, DFS and RFS for prognostic values.

Unavailable information from included studies will be sought from authors by email. Any discrepancies will be resolved by consensus between AR and RR.

### Assessment of methodological quality

The methodological quality of primary studies will be assessed by a revised tool devised for QUADAS-1 quality assessment.<sup>50</sup> The defined questions will be answered as yes, no or unclear, and the score of each article will be calculated. Two authors (AR and RR) will initially complete the pilot QUADAS-1 items in three studies. If the authors do not agree, we will refine the tool, and then the updated tool will be applied to complete the assessment for all included studies. Disagreements will be resolved by consensus.

### Assessment of heterogeneity

To investigate heterogeneity, we will include the study design (prospective or retrospective and year of publication), population characteristics (gender, ethnicity, age, disease types and stage distribution), test characteristics (cut-off value, test type and the total number of tests per screening round) and versions of reference standards as our study-level variables. We will test these study-level covariates in the bivariate model in the common threshold or add them to the Rutter and Gatsonis HSROC model to evaluate the heterogeneity in the test threshold, diagnostic accuracy and the shape of curves. The likelihood ratio test will be used to determine the statistical significance of the covariates included in the models.<sup>51</sup>

### Assessment of reporting bias

The publication bias will be assessed by funnel plots (ie, plots of study results against precision) and Begg's and Egger's tests.

### Statistical analysis and data synthesis

#### Descriptive analysis

All included studies will be overviewed and presented in two separate tables. The first table will provide details on the study quality according to the review-specific QUADAS-1 tool. The other table will include the study design, participants, test characteristics, results, outcomes and reference standards. The following test characteristics will be extracted into 2×2 tables (TP, FP, TN and FN) for all included studies. Study-specific estimations of sensitivity and specificity with 95% CI will be displayed in forest plots using Review Manager (V.5.2). These graphical displays will reveal the variations in accuracy among the studies and the different types and

brands of the index test. The collected data about the outcomes such as DFS, OS, RFS and HR with 95% CI will be used for calculating the prognostic value of the index test.

### Inferential statistics

All the included studies will be synthesised after a systematic review. Study-specific HR, sensitivity and specificity estimates will be pooled using a fixed effect model, if no significant heterogeneity exists; otherwise, a random effect model will be applied. The extent of heterogeneity throughout the studies will be checked using the  $\chi^2$  test and  $I^2$  test;  $p=0.10$  and/or  $I^2 >50\%$ , indicating significant heterogeneity. To evaluate the sources of heterogeneity, subgroup analysis and meta-regression will be performed.

The potential for publication bias will be assessed using funnel plots, Begg's rank correlation method and Egger's weighted regression method. A  $p$  value of  $\leq 0.05$  will be considered to be statistically significant.

All the mentioned analyses will be performed using STATA V.11.1 software (StataCorp LP, College Station, Texas, USA).

### Sensitivity analysis

A sensitivity analysis will be conducted to test the impact of the results with respect to the methodological quality items rated by the QUADAS-1 tool criteria. We will also implement sensitivity analyses to explore the impacts of methodological quality and sample size on the robustness of review conclusions. Meta-analyses will be repeated after excluding studies with lower methodological quality and studies with sample sizes much larger than those of other studies. Sensitivity analyses will be reported with a summary table, and reviewed conclusions will be interpreted by making comparisons between the two meta-analyses.

### Subgroup analyses

Subgroup analyses will be conducted according to the region, gender, quality, sample size and cancer subtype.

## DISCUSSION

Despite the comprehensive understanding of the biology of RCC and recent advances in novel therapeutic strategies, the lack of molecular biomarkers for the early detection and classification of RCC and the poor prognosis for these patients are major complications. miR-21 is a tumour marker that is upregulated in RCC and in several tumours. In RCC, miR-21 is involved in cellular mechanisms such as growth, apoptosis, cell cycle, invasion and migration of tumour cells.

Our systematic review will clarify the prognostic and diagnostic accuracy of miR-21 in patients with RCC, which would allow patients, clinicians and researchers to determine the diagnostic performance of miR-21 for the detection of RCC, particularly early stage RCC, and the

potential role of this marker in the prognosis of patients with RCC. This systematic review will also help policy-makers and guideline developers in the management of patients with RCC.

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**Contributors** MM, ZM, AR and AAK contributed to the concept and study design. AAK and AR developed the search strategies and contributed to the implementation and analysis of the studies. AR contributed to the initial drafting and critical revision and approved the manuscript for submission. RR and SB revised the manuscript. AR and RR will also screen potential studies, extract data and assess their quality. Any discrepancies will be resolved by consensus between the two authors.

**Competing interests** None declared.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** All recorded data from the data extraction process will be available on request to the extent that they are not included in the systematic review article.

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